Targeted genome editing approach for the treatment of β-thalassemia

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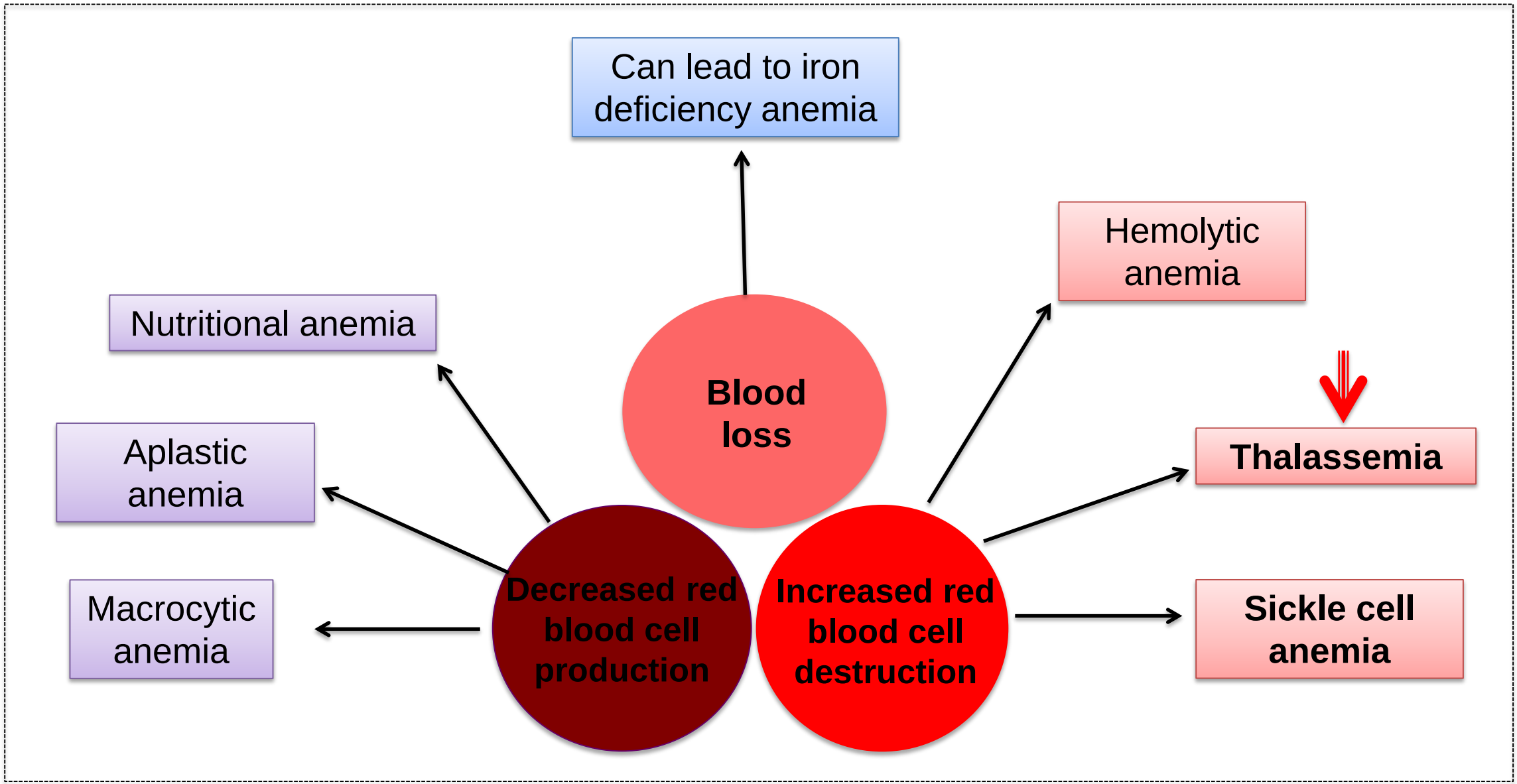
- Generation of non-deletional HPFH mutations to reactivate HbF using genome-editing approach.

- Establishing isogenic parental and genetically modified iPSC lines for various β-thalassemia mutations using CRISPR/Cas9 approach for drug screening.

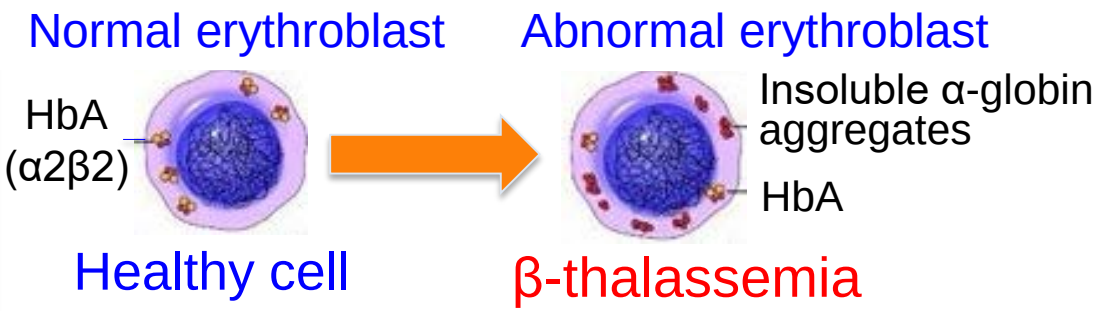
Types of anemia



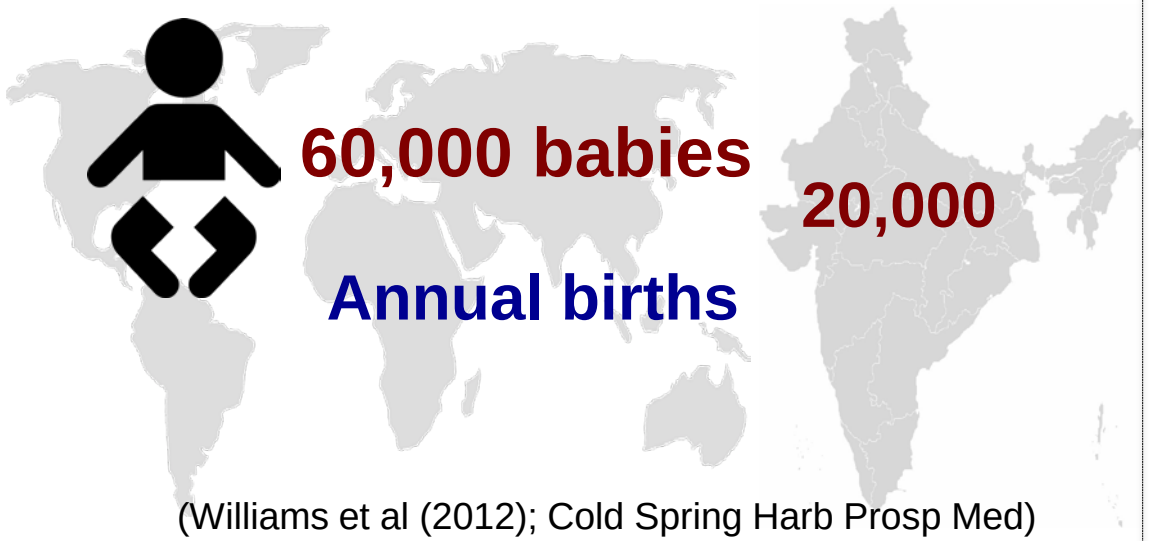
(Modified from https://infograph.venngage.com)

β-thalassemia (BT);Global health burden

β-thalassemia caused by mutations in the β-globin gene



Significant global burden



(Williams et al (2012); Cold Spring Harb Prosp Med)

High morbidity and mortality

Anemia

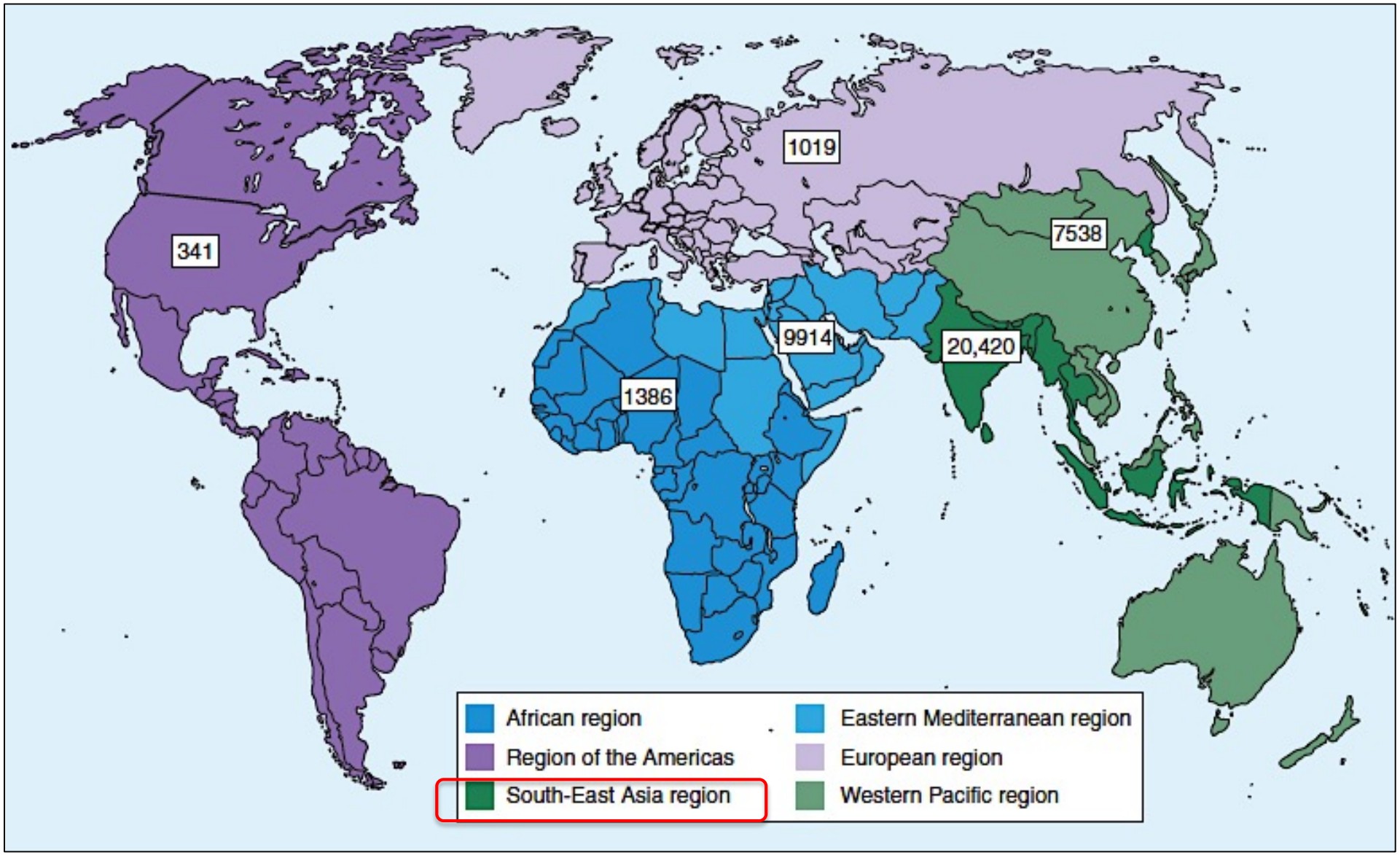
Pain

Early death

Heavy burden of patient care

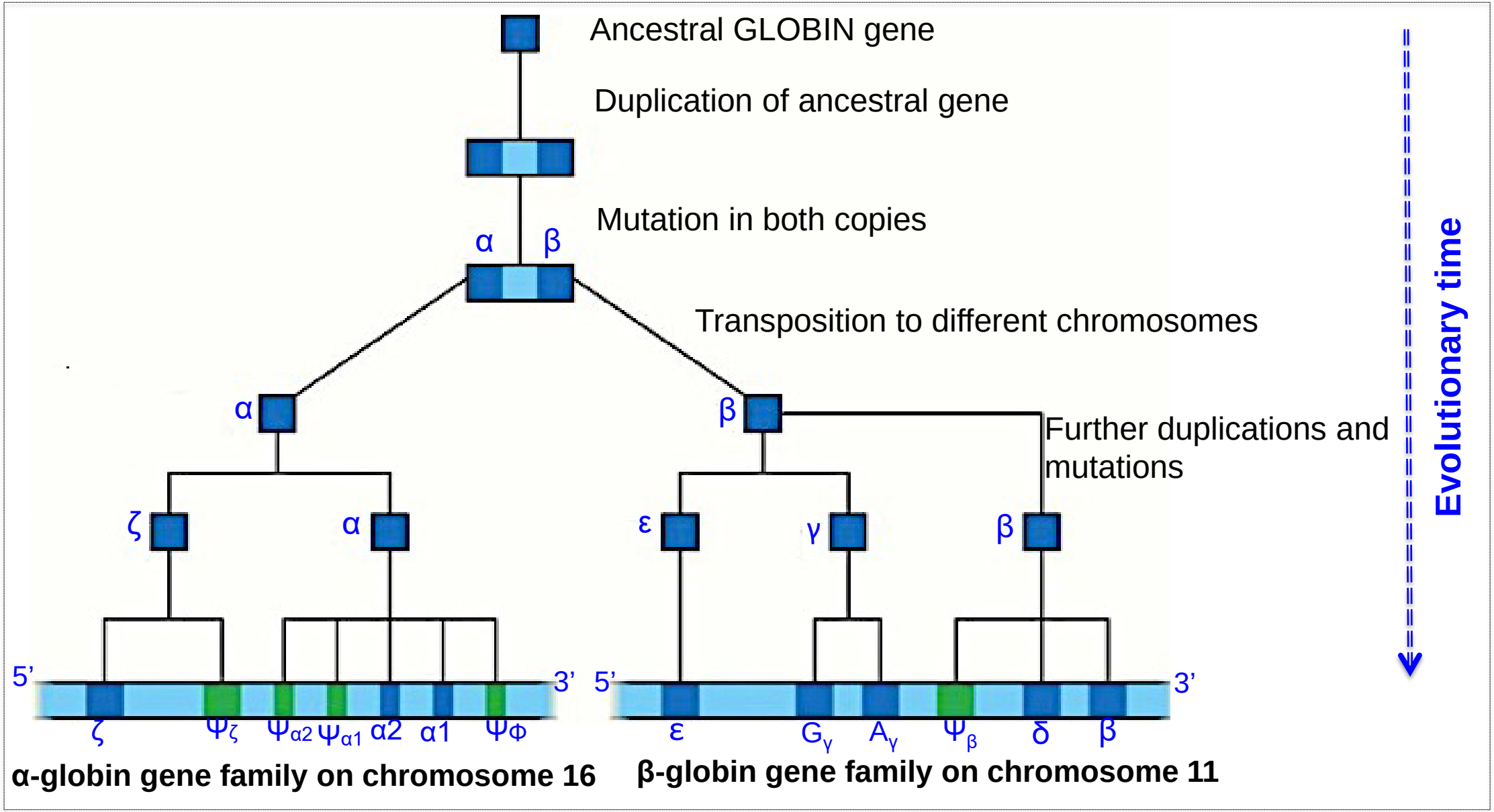
Frequent transfusions & hospitalizations

Estimated annual affected births of children with β-thalassemia in different regions of the world



(Colah et al 2010; Exp Rev Hematol &
Piel et al 2016; Hematol Oncol Clin N Am)

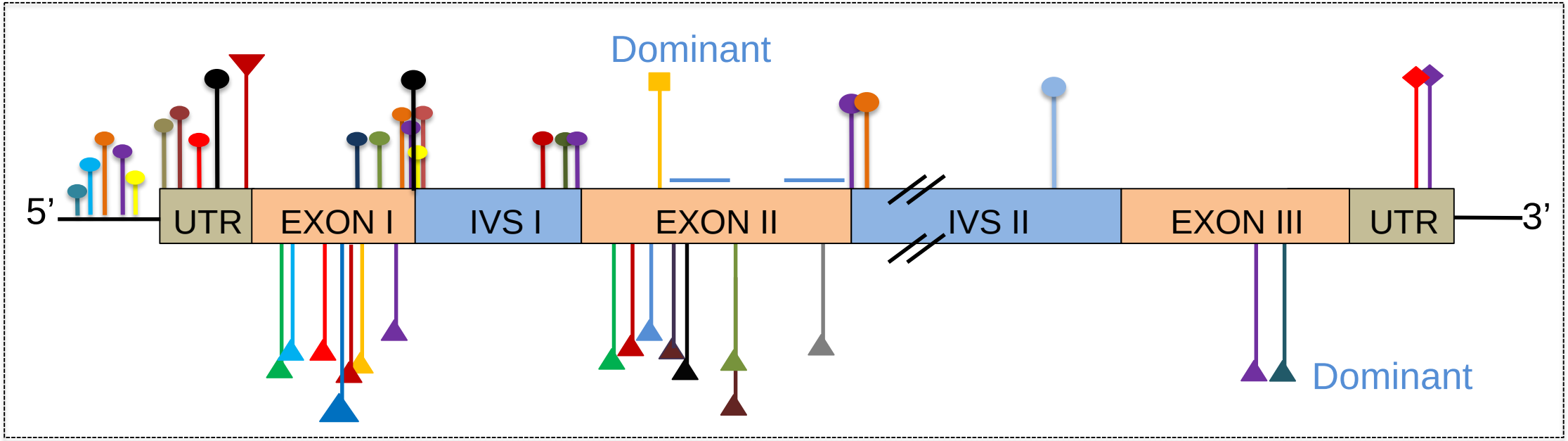
Evolution of GLOBIN gene



Evolved from one common ancestral globin gene which duplicated and diverged

β-thalassemia; a heterogeneous genetic disorder

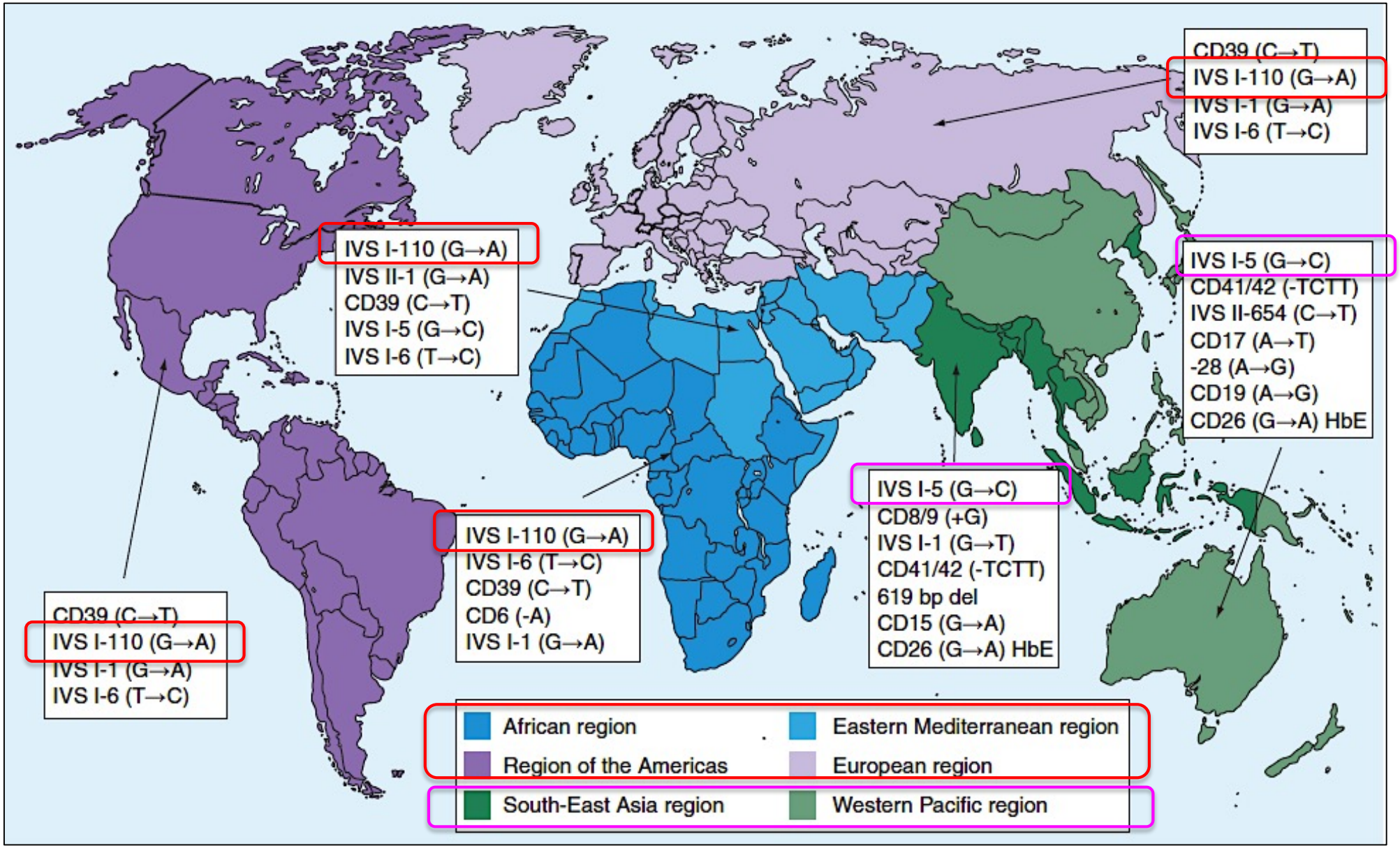
- It is caused by more than 200 different mutations in the β-globin gene.



- These mutations lead to reduced or no synthesis of β-globin molecule leading to surplus production of unpaired α-globin chains that can deposit within erythroid precursors.

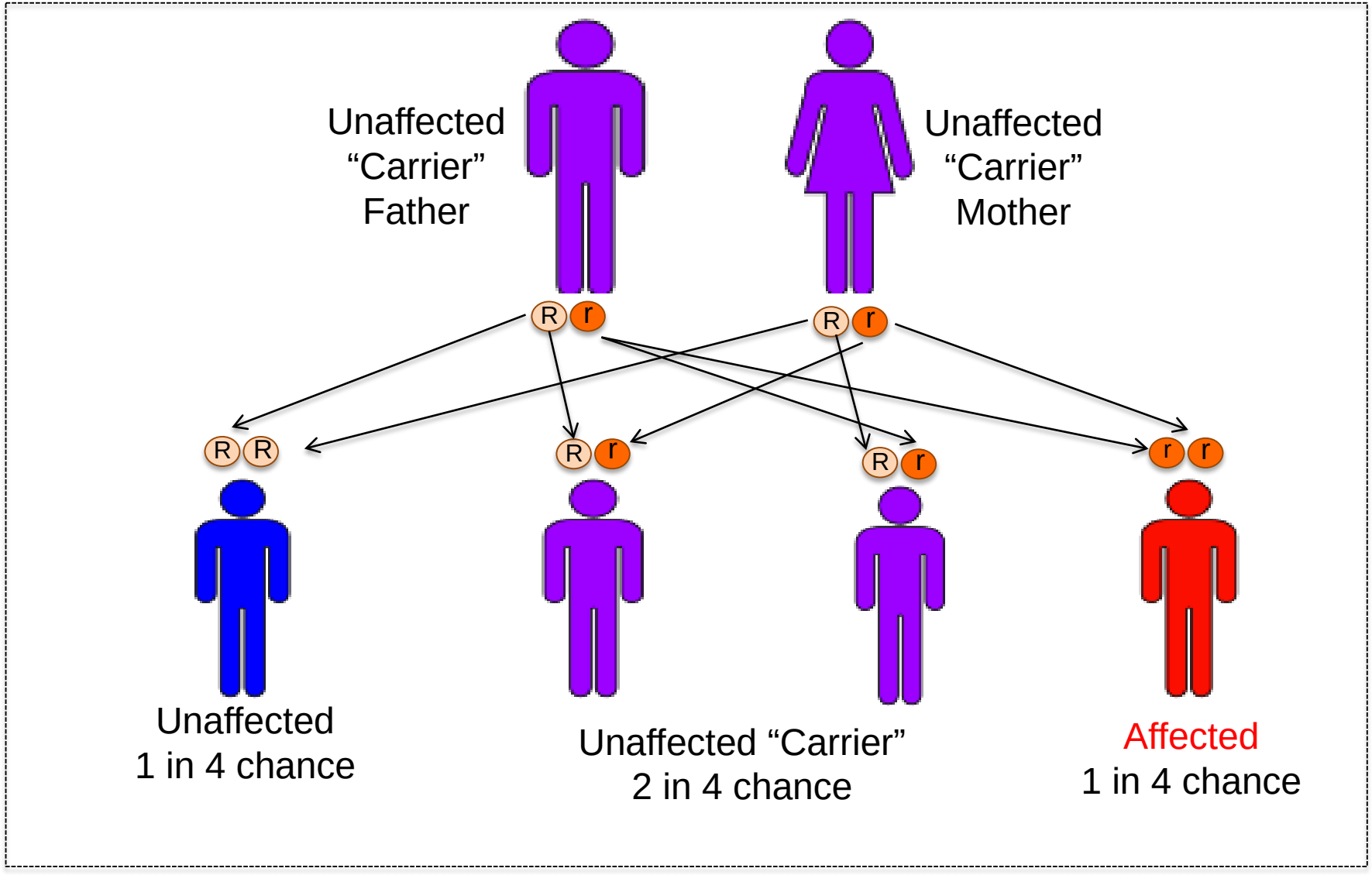
- The maturation of these precursors gets impaired by deposition of these α-globin chains causing **inadequate generation of RBCs**.

Common β-thalassemia mutations in different regions of the world

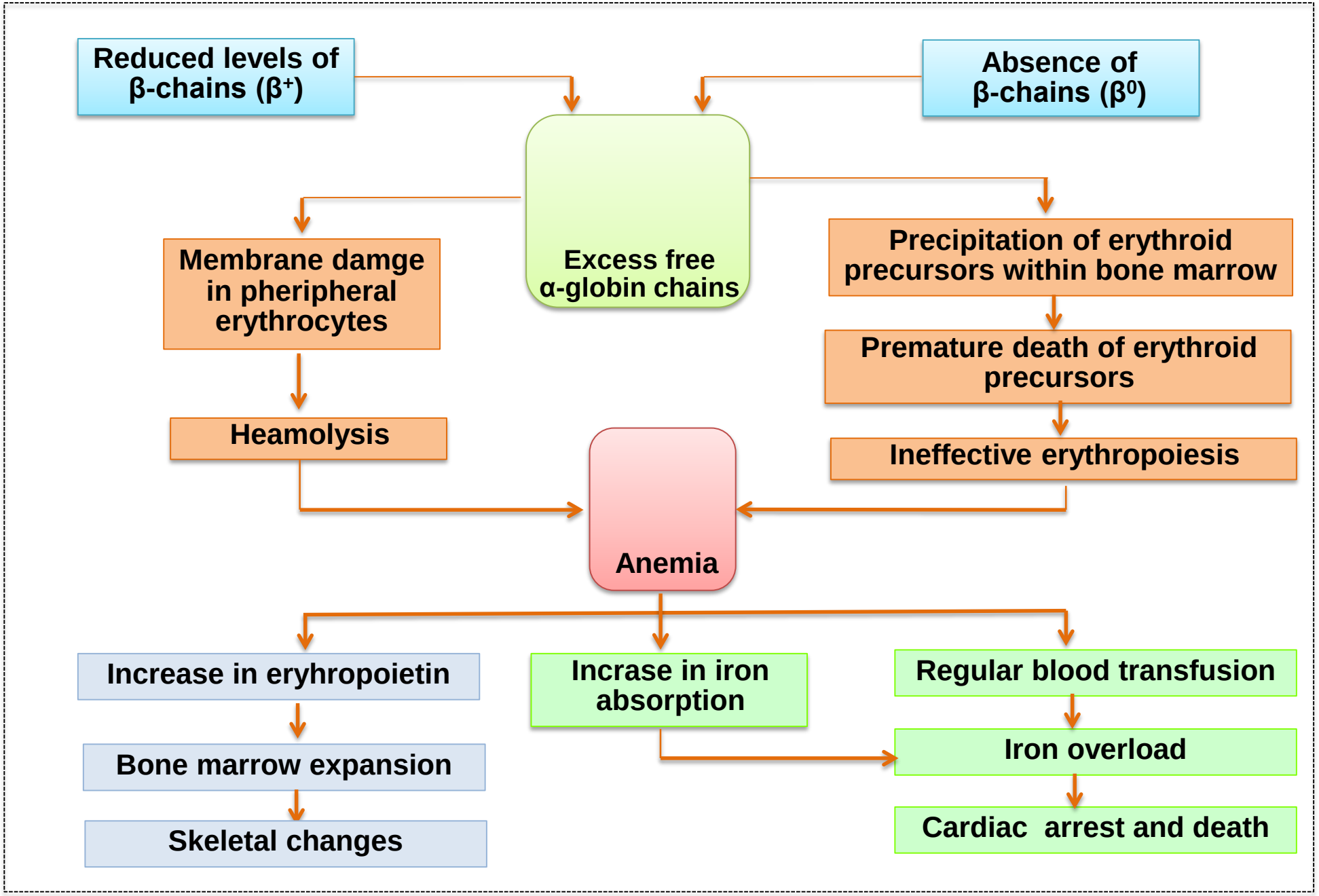


(Colah et al 2010; Exp Rev Hematol)

Inheritance of thalassemia; How the trait is passed on



Pathophysiology of β-thalassemia



(Modified from Madeleine et al 2017)

Current treatment for β-thalassemia

- Treatment of β-thalassemia is still largely dependent on supportive care with **regular red blood transfusions and iron chelation**

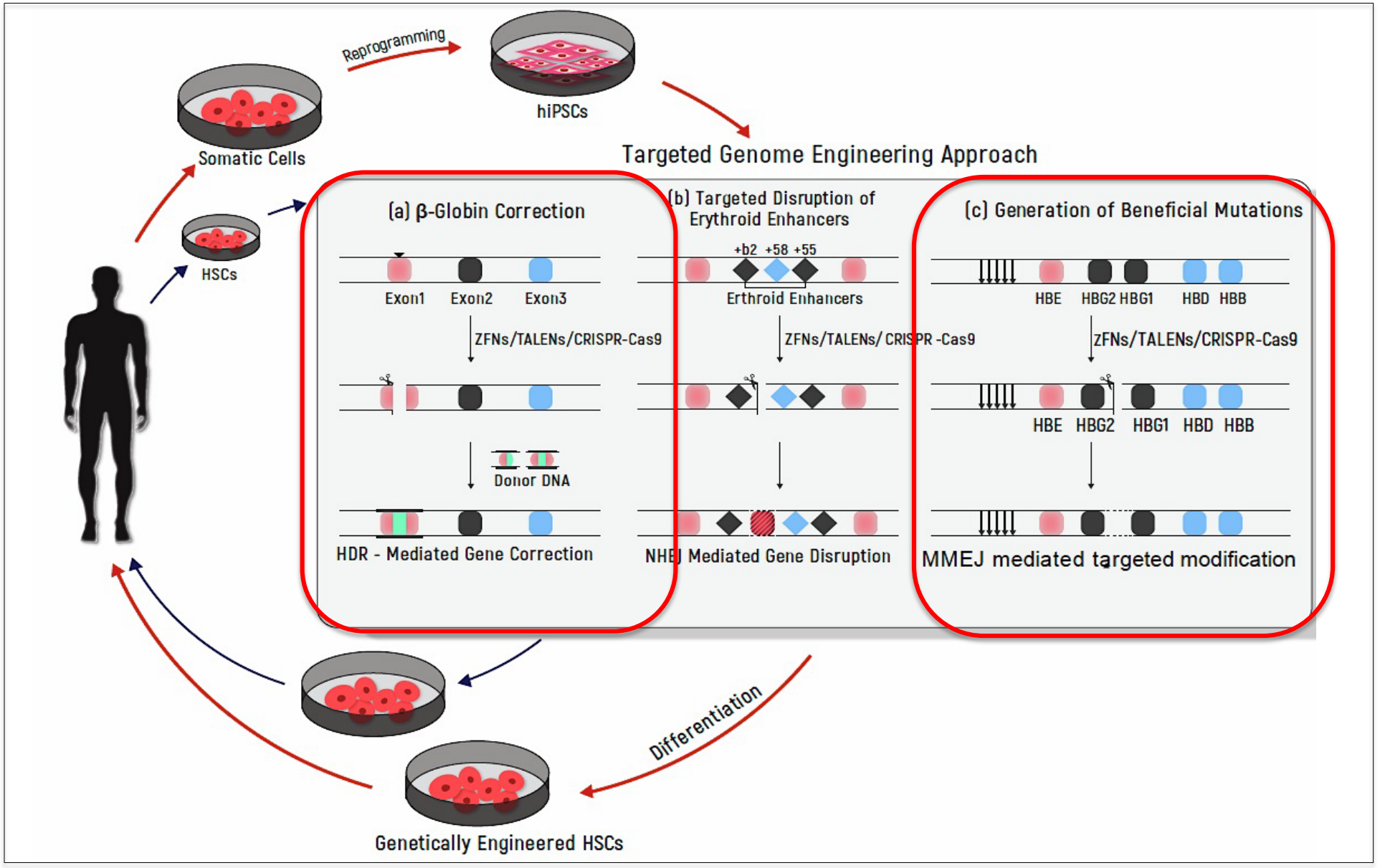
- The only definitive cure at present for this disorder is hematopietic stem cell (HSC) transplantation.

- However, there are numerous complications associated with this procedure, including difficulty in finding a HLA-matched donor, graft-versus-host disease and graft rejection after transplantation.

- Lentiviral based gene therapy has recently provided alternative approach for treatment of β-thalassemia. Safety concerns of viral vectors.

- **Evolutions in genome editing with advances in induced pluripotent stem cells (iPSCs) and HSCs provide realistic opportunities to tackle the β-hemoglobin disorders**.

Targeted genome editing approach for the treatment of β-thalassemia

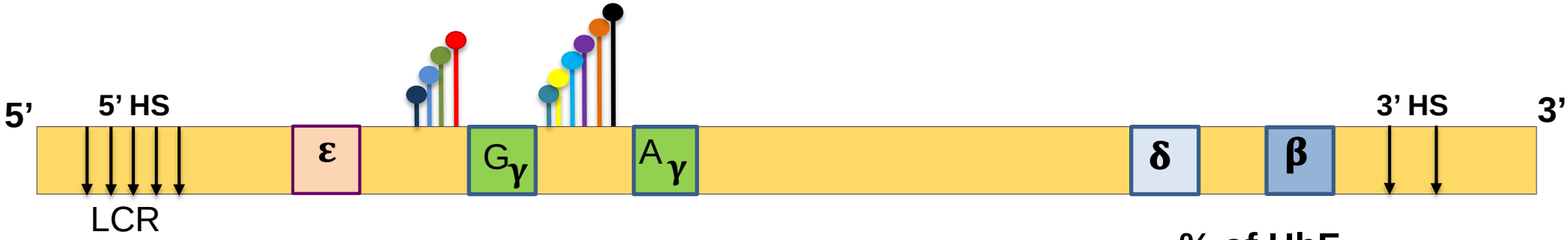


(Lohani N, Bhargava N, Munshi A, Ramalingam S (2018). *J Cell Physiol*)

Hereditary persistence of fetal hemoglobin (HPFH): "A Natural way to enhance HbF expression"

- Expression levels of HbF is reduced postnatelly to <1% of total hemoglobin.
- Individuals who inherited genetic mutations **in the upstream promoters region of a γ-globin genes** that prevent the fetal to adult Hb switch, express **significantly enhanced HbF** throughout their life - **HPFH**.
- **Clinical symptoms of SCD and β-thalassemia are alleviated in patients who inherited HPFH genotype** (Steinburg et al 2014, Blood).
- Around 20% of HbF appear sufficient to prevent clinical crises (Powars et al 1984; Blood)
- Hence, introducing these naturally occurring HPFH genetic mutations using targeted genome engineering will alleviate the symptoms of β-hemoglobin disorders.

Nondeletion type of hereditary persistence of fetal hemoglobin (HPFH)

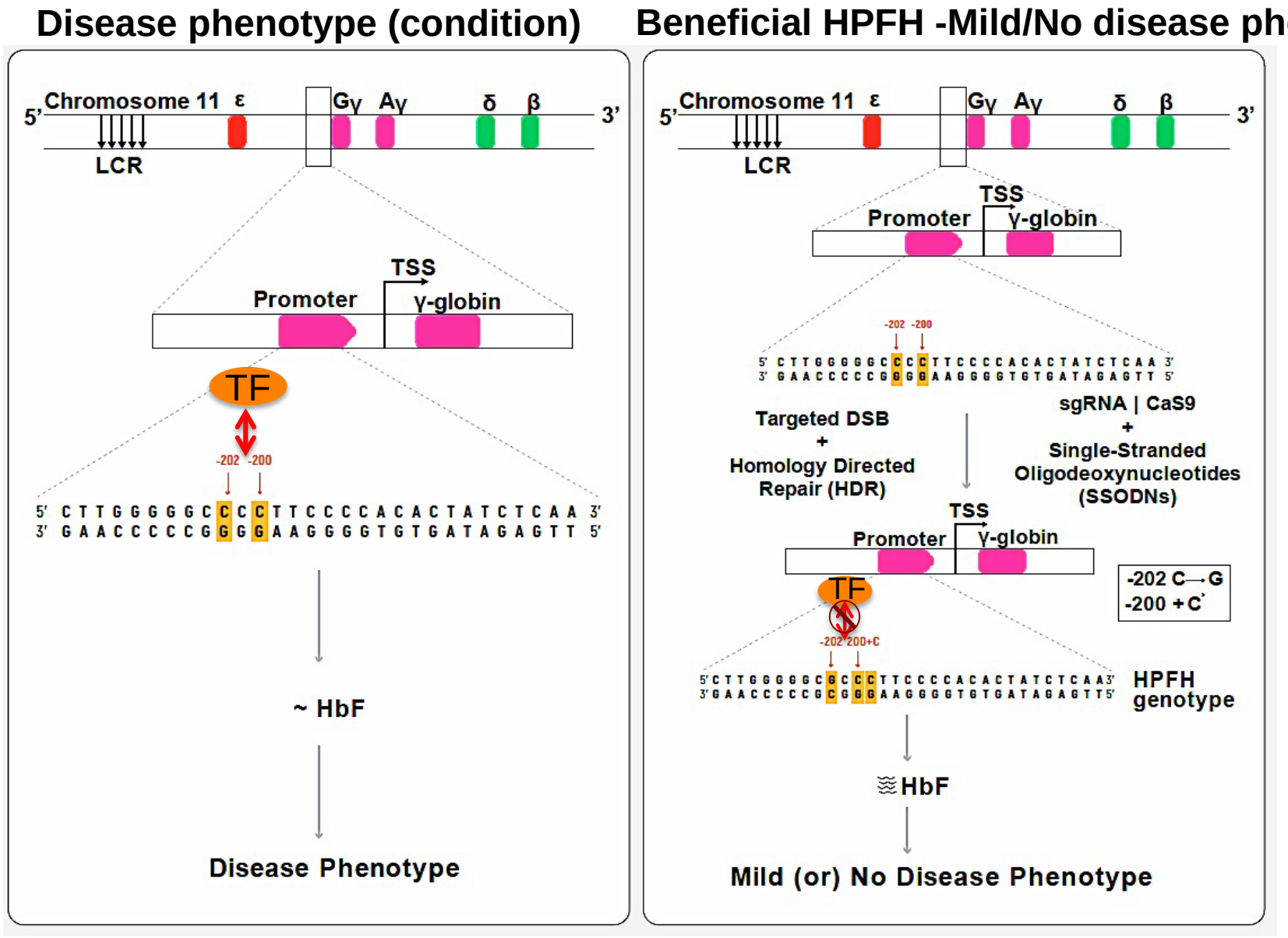


	% of HbF
Gγ -202 C>G (Black)	18-23
Gγ -200 +C (Tunisian)	27-30
Gγ -175 C>G (British/Sardinian/Black)	18-21
Gγ -114 C>T (Japanese)	11-14
Gγ -114 C>G (Australian)	8-10
Gγ -114 C> (Algerian)	1-2
Aγ -202 C>T (Black)	2.5-4
Aγ -198 T>C (British)	7-9
Aγ -196 C>T (Italian/Chinese)	14-16
Aγ -195 C>G (Brazilian)	11-14
Aγ -117 G>A (Greek/Black)	3-5

(Forget 1998; *Ann N. Y Acad Sci* & Angestiniotis et al 2013 *Preven hemo dis*)

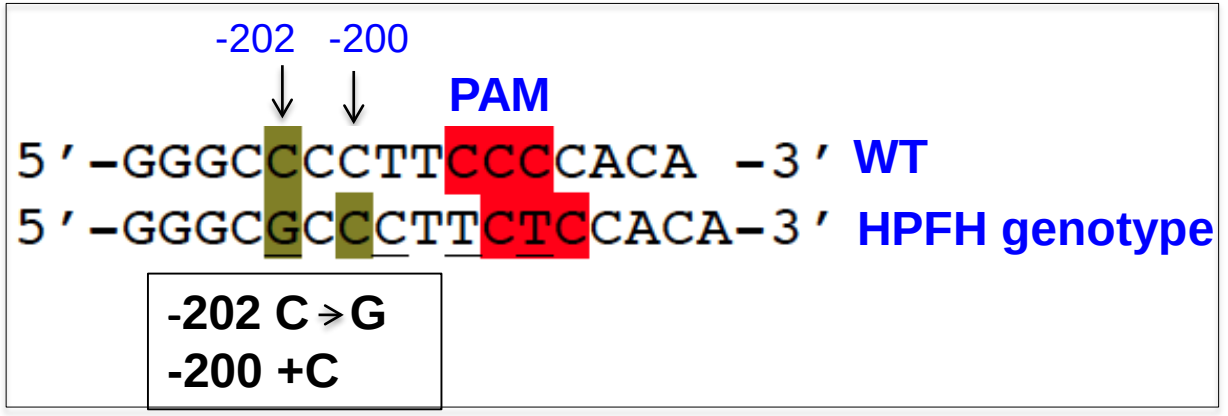
Generation of non-deletional HPFH mutations to reactivate HbF using genome-editing approach

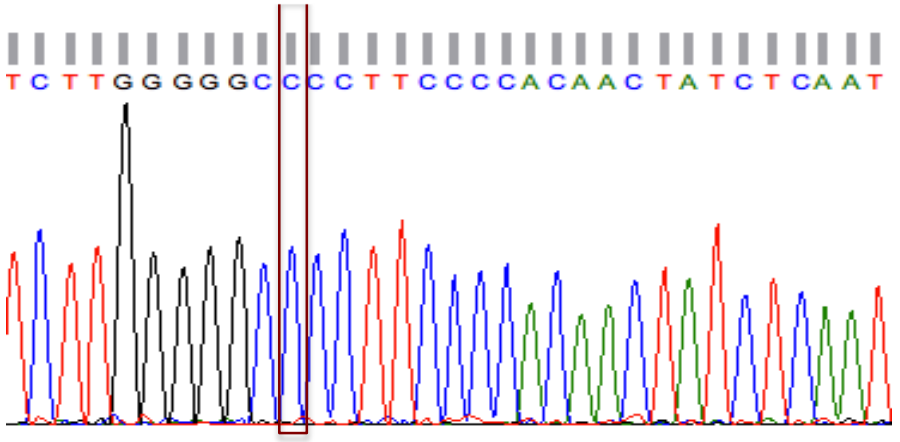
Hypothesis



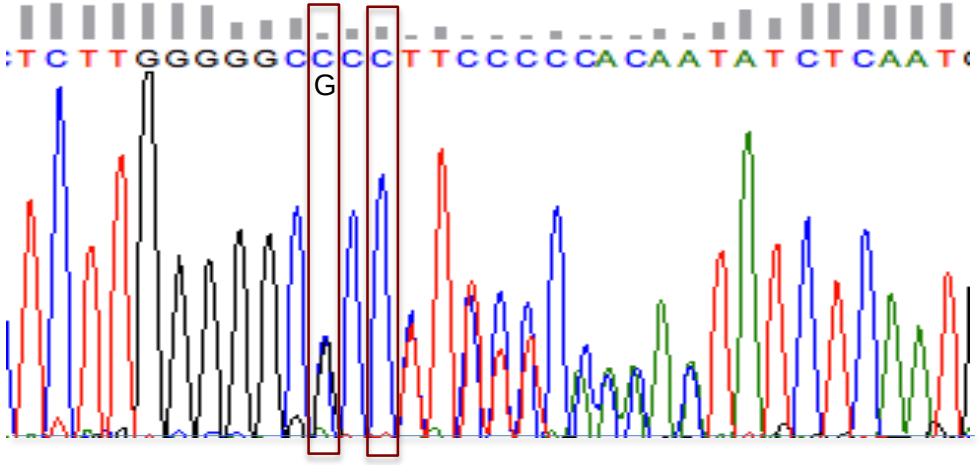
Clinical symptoms of SCD and β-thalassemia can be alleviated with beneficial HPFH genotype

Sequencing results confirmed successful generation of non-deletional HPFH genotype

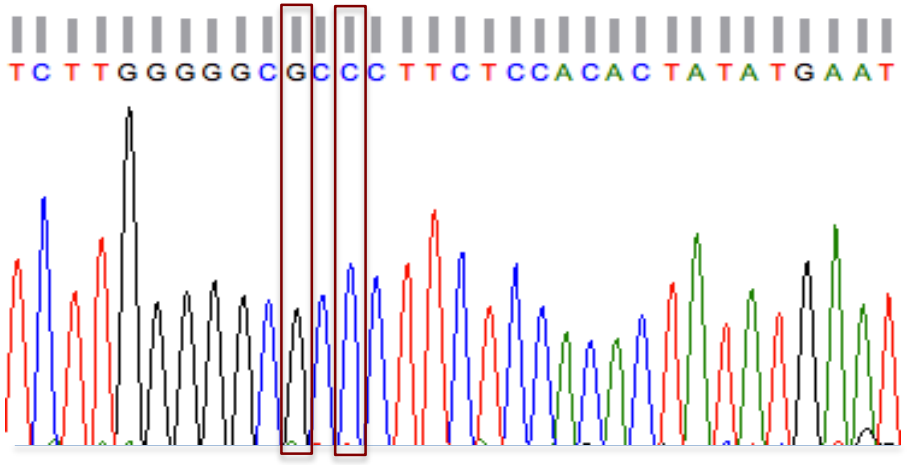




WT-HBG2



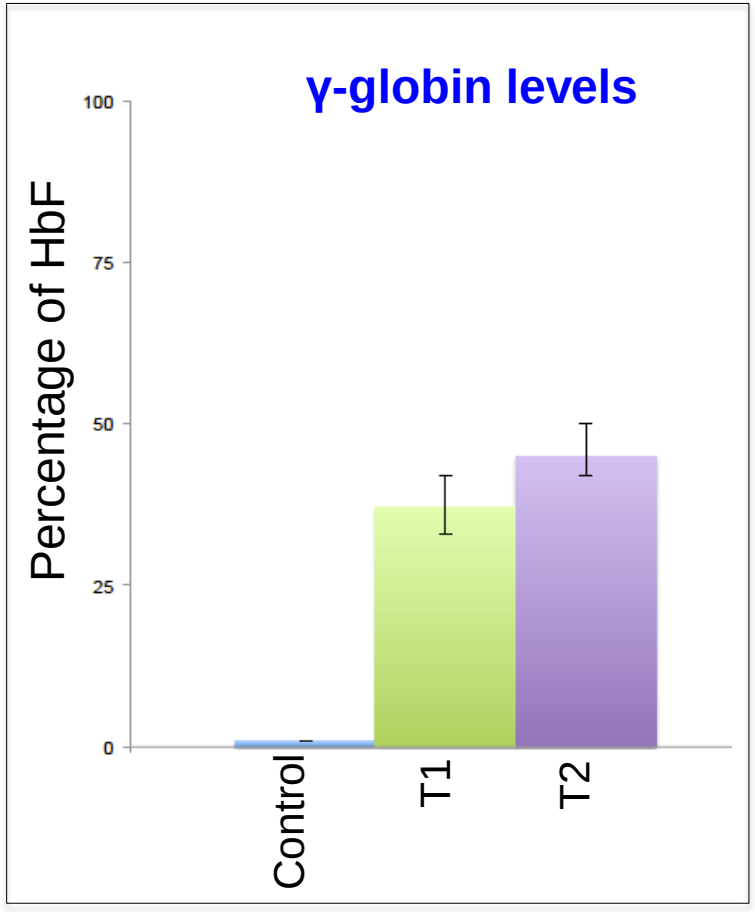
HBG2-HPFH-genotype (Monoallelic)

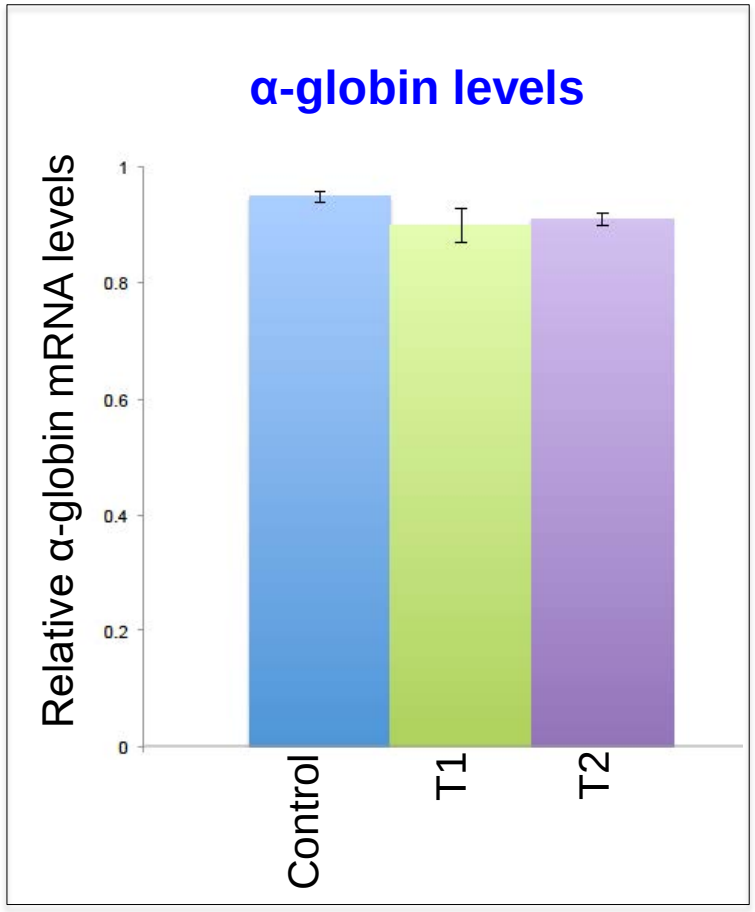


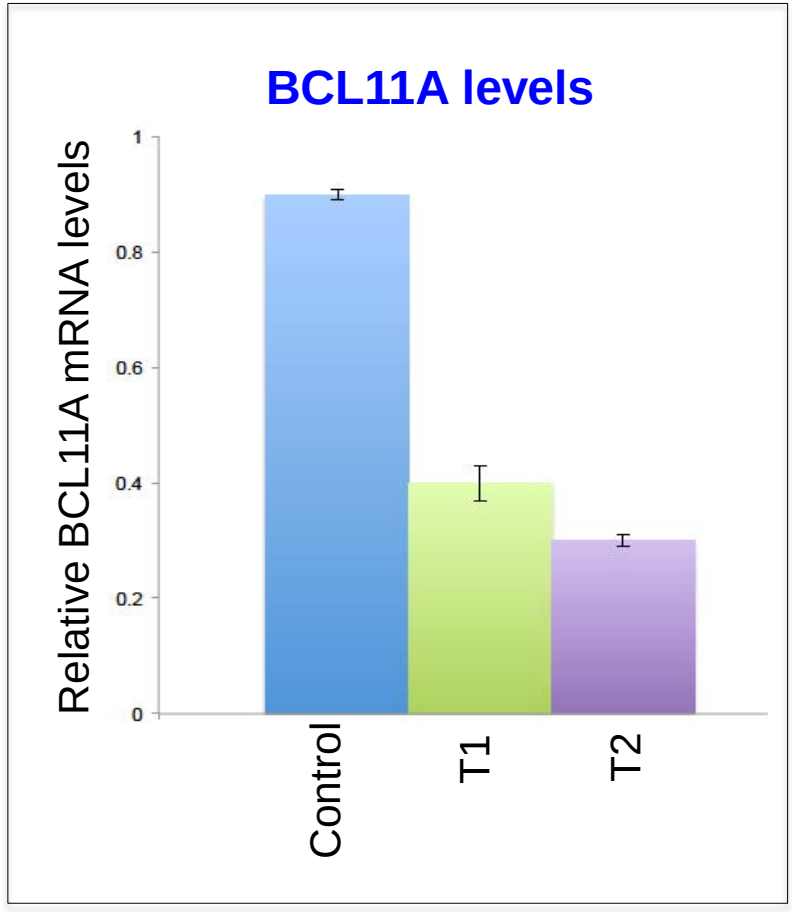
HBG2-HPFH genotype-Biallelic

Generated beneficial HPFH genotype in erythroid cells using genome-editing approach

qRT-PCR analysis of HPFH mutant cells

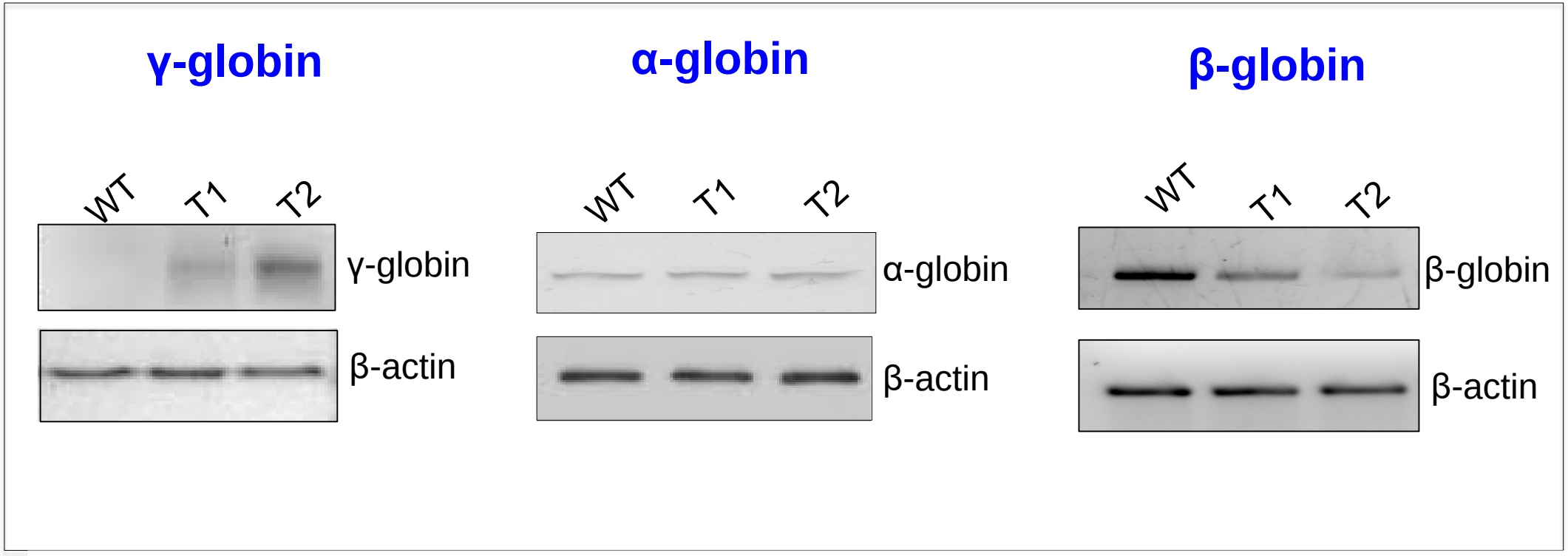






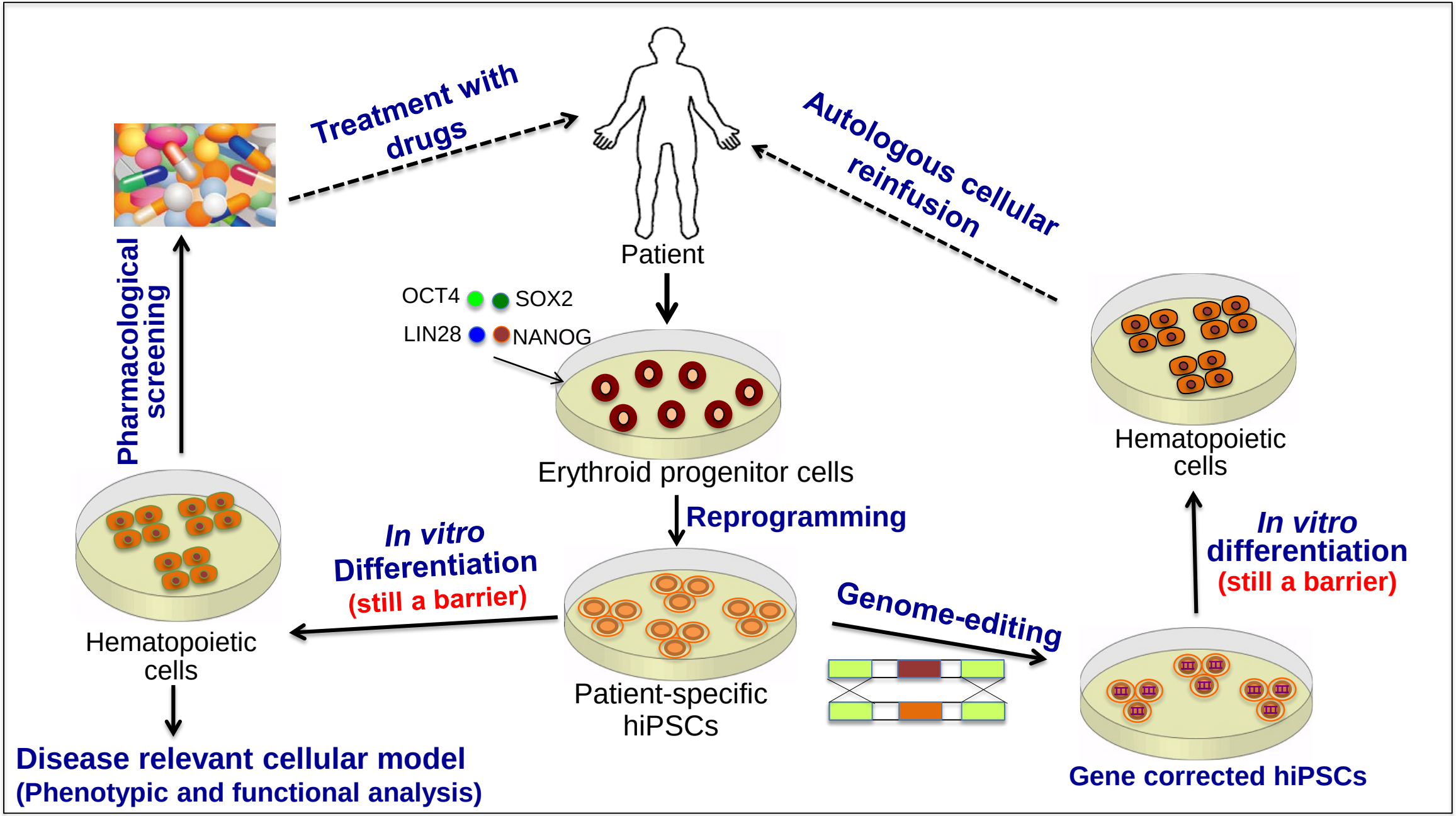
Beneficial HPFH genotype generated using genome-editing approach enhanced γ-globin transcript levels

Western blot analysis of HPFH mutant cells



Beneficial HPFH genotype generated using genome-editing approach enhanced HbF levels

Establishing isogenic parental and genetically modified iPSC lines for various β-thalassemia mutations for drug screening



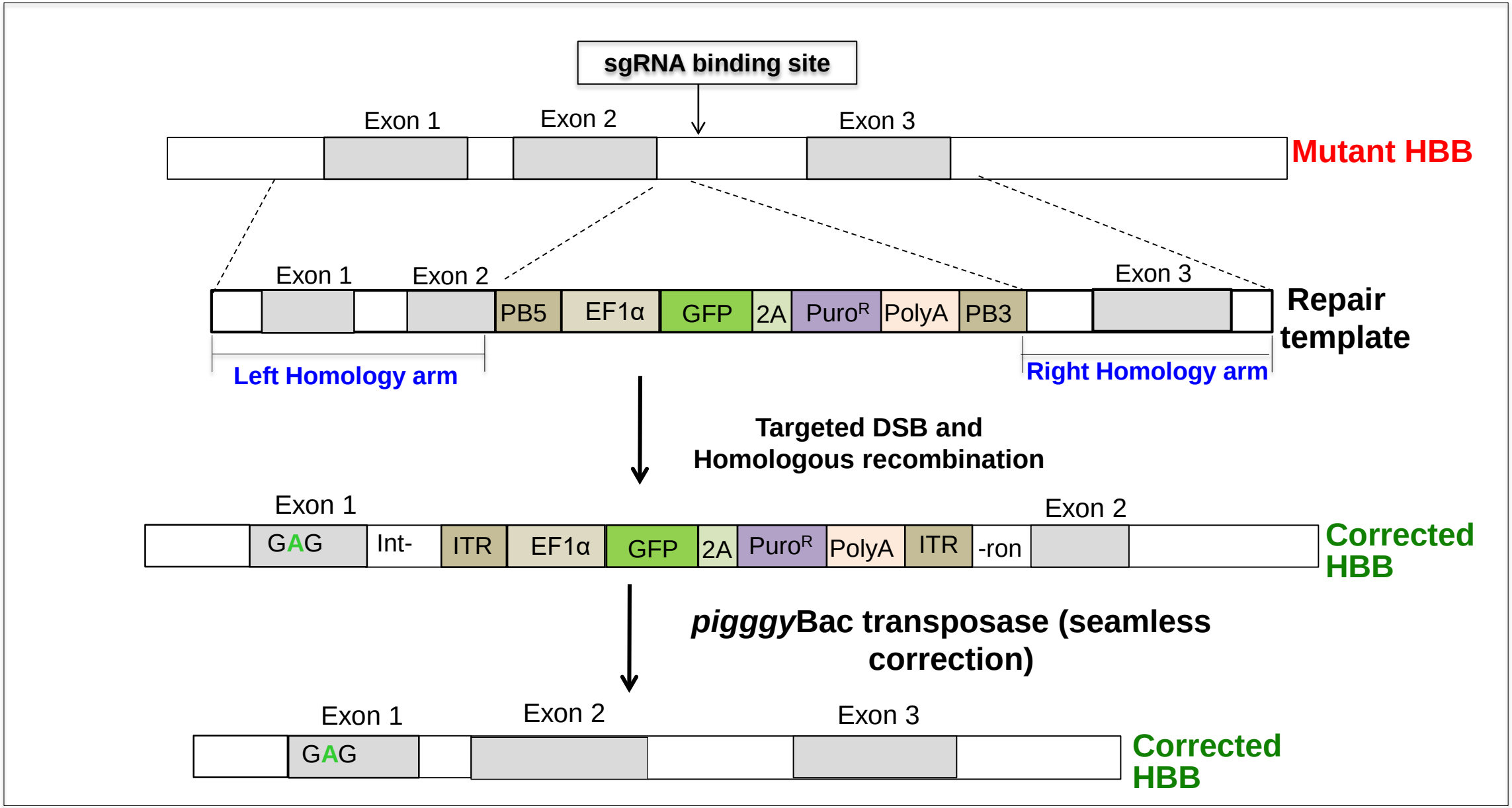
Presence of common and rare β-thalassemia mutations in India

S.No.	Mutations	%*
1	IVS 1-5 (G>C)	65
2	Codon 15 (TGG>TAG)	10
3	Codon 41/42 (-TCTT)	8
4	Codon 26 (G>A) HBE	3
5	Frameshift codon 8/9 (+G)	3
6	IVS II 837 (T>G)	3
7	HbE (GAG>AAG)	2
8	PolyA (T>C)	2
9	Codon 5 (C>T)	2
10	Codon 30 (G>C)	2
11	Codon 16 (-C)	2
12	619 bp deletion	2

S.No.	Mutations	%*
13	Cap site 1	0.4
14	IVS II 1	0.4
15	HBB C112-122 DDT	0.3
16	Codon 17 (A>T)	0.3
17	IVS 1-1 (G>A)	0.3
18	IV II 5 (G>A)	0.3
19	Codon 106 (GTG>CGG)	0.1
20	C.93-94 INS CTG Mutation in HBB	0.1
21	HBB 166-166 DD A	0.1
22	Codon 1-5 (G>C)	0.1

***Percentage was calculated based on 1000 β-thalassemia patient genetic data**

Schematics showing genetic correction of various β-thalassemia mutations in patient-derived iPSCs using single donor DNA



Conclusion

- We have shown genome-editing can be used to generate non-deletional HPFH mutations in human erythroid progenitor cells, which leads to reactivation HbF

- We have established isogenic parental and genetically modified iPSC lines for various β-thalassemia mutations using CRISPR/Cas9 approach.

Acknowledgements

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DST-Science and Engineering Research Board (SERB)

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Thank you